Intravascular Aggregation and Adhesiveness of the Blood Elements Associated with Alimentary Lipemia and Injections of Large Molecular Substances

Effect on Blood-Brain Barrier

By Chester F. Cullen, M.D., and Roy L. Swank, M.D., Ph.D.

Observations on the intact circulation of the hamster reveal that following a meal high in fat content there occurs increased adhesiveness and aggregation of the red blood cells and sometimes clumping of the platelets, accompanied by slowing of the circulation. These changes are reversible, the circulation resuming a normal appearance with complete clearing of the lipemia. Similar circulatory changes can be produced by the injection of large molecular solutions. These circulatory alterations, when severe enough, cause pathologic changes in the blood-brain barrier. The significance of these findings and their possible relationship to multiple sclerosis and vascular thrombosis are discussed.

Geographic and nutritional surveys\(^1\),\(^2\) have suggested that the geographic variations in the incidence of multiple sclerosis are due, at least in part, to the amount of fat consumed; a high fat consumption is associated with a high incidence of multiple sclerosis and a low fat consumption is associated with a low incidence of the disease. It is felt that a high fat intake is not the cause of multiple sclerosis, but rather precipitates or accelerates it in susceptible individuals. In accordance with this concept multiple sclerosis has been treated with a low fat diet for the past four and one-half years with the result that the number and severity of exacerbations of the disease seem to have been decreased.\(^3\)

In an attempt to determine the mechanism by which the fat intake influences multiple sclerosis, studies have been made of the effects of large fat meals on the blood of dogs and humans. Six to nine hours after large fat meals the red blood cells observed in vitro in dark-field illumination have a tendency to aggregate, become adhesive to one another and be distorted.\(^4\) These changes in the suspension stability of the blood occur about an hour after the peak of the alimentary lipemia and finally disappear 9 to 12 hours after the fat meal when the lipemia clears. These alterations in the suspension stability are accompanied by changes in the sedimentation rate and by alterations of the plasma protein patterns in paper chromatograms.\(^5\)

The present study was undertaken to determine the effect of high fat meals on the intact circulation. In addition we have studied the previously reported circulatory changes produced by intravenous injections of substances of high molecular weight,\(^6\) and observed the effects of these circulatory changes and those due to high fat meals on the blood-brain barrier. A preliminary and partial report of the changes produced by large fat meals has been published.\(^7\) In the present paper the completed study will be presented.

Material and Methods

Golden Syrian hamsters weighing 70 to 100 Gm. were used throughout the study. The control
hamsters consumed a diet of mixed grain, vegetable greens and foxchow (approximately 20 per cent of the total calories of foxchow are furnished by fat; the vegetable greens and mixed grain which made up a large part of the diet contain very little fat). Many of the experimental hamsters received a diet containing approximately 50 per cent of the calories as fat for one to three weeks prior to observation. Other experimental hamsters received the normal control diet prior to the single fat meal given on the day of observation.

On the day of observation, a high fat meal (2 to 15 Gm. per kilogram of body weight) in the form of 35 per cent cream was fed via stomach tube. At variable intervals after the fat meal the animals were anesthetized with 20 per cent urethane intraperitoneally and one cheek pouch was inverted and prepared for visualization by the method of Fulton, Jackson and Lutz. In most cases one membrane of the pouch was removed to improve the visualization of the blood elements, but for control purposes a number of pouches were studied with the membranes intact to determine the effect of the increased trauma and exposure occasioned by the removal of one membrane. The pouch was immersed in Ringer’s solution in a specially designed water bath and maintained at a temperature of 37.5° C. Observations were made at magnifications of 750 to 1350×, and for the cinematomicrographs a 50× water immersion objective was used. The Kodachrome cinematomicrographs were taken at 32 frames per second. In a number of hamsters a small polyethylene tube was placed in the femoral vein so that injections could be made and frequent blood samples could be obtained. The blood samples were observed in darkfield illumination to determine the chylomicron counts, and in six of the fat-fed hamsters platelet and leukocyte counts were made. For control purposes a number of animals were studied without inserting the tube.

This study is based on observations in 105 hamsters. Thirty of these were on the normal diet, some fasting, the others nonfasting; 35 were fat-fed; 22 received injections of high molecular weight substances (gelatin, dextran); and 18 were subjected to miscellaneous procedures (injections of egg albumin, thromboplastin, and histamine liberators; and anaphylactic shock). Microscopic observations on each animal lasted for a period of 3 to 10 hours.

In 24 of our hamsters a saturated solution of trypan blue (approximately 13 per cent) was injected intravenously at the termination of the experiment to determine if the alterations of the circulation were sufficient to damage the vessels of the brain. One milliliter was injected, and was followed in 5 to 10 minutes by the injection of an additional 1 ml. Of this group 6 had received intravenous dextran, 7 had been given high fat meals, and 11 served as normal controls. The control animals were subjected to the same surgical trauma as the others, and the injections of trypan blue were made at varying intervals up to five and three quarter hours after exposing the pouch.

RESULTS

A. Circulation in the Cheek Pouch of Normal Hamsters

In none of our control studies of the cheek pouch of the hamsters was significant stickiness or aggregation of the red blood cells noted. (See figure 3A.) A variable amount of adhesiveness of the white blood cells to the walls of the blood vessels and clumps of platelets were occasionally observed. The stickiness of the white blood cells to the vessel walls usually increased after three or more hours of exposure of the pouch at which time the leukocytes could sometimes be seen migrating through the vessel wall into the surrounding tissue. Petechial hemorrhages also began to appear about this time. Groups of two to three red blood cells in rouleau formation were occasionally observed in normal pouches. These were present only in vessels with a slowly moving stream of blood; the individual cells were clearly distinguishable and readily separated from one another when the flow of blood changed direction or was interrupted by a more rapid flow in anastomosing vessels. Even when the flow was greatly slowed or completely stopped by the production of anaphylactic shock or of shock due to the injection of the histamine liberator 48/80, no increased tendency to adhesiveness or aggregation of the red blood cells was noted. It has also been shown in the rat and in the hamster that circulatory arrest, per se, does not cause aggregation of the red blood cells.

B. Circulation in the Cheek Pouch of Lipemic Hamsters

It was found that the hamsters usually developed a greater alimentary lipemia if they had been on a high fat diet for a period of several days. Most of our animals had been on such a diet for one to three weeks prior to observation and frequently this was supplemented by a daily feeding of cream for 2 to 4 days prior to visualization of their pouches. We have, however, observed a significant lipemia followed by the changes now to be described.
Fig. 1. The chylomicon curve represents the average in 19 hamsters which had counts done at sufficiently frequent intervals to determine the nature of the visible lipemia curve. The + sign indicates the time at which increased adhesiveness of the red blood cells appeared in each of the animals. The 0 sign indicates the time at which the circulation started to revert toward normal in each of 11 of these hamsters which were observed for a long enough time to note the reversal. In most of the animals increased adhesiveness appeared four to seven hours after the fat meal; and the circulation began reversal toward normal 7 to 10 hours after the large fat meal in most instances.

following a single fat meal without prior large fat feedings.

On the day of visualization the pouch was exposed three to six hours after the fat meal. No change was observed in the circulation during the period of increasing visible lipemia. When the chylomicon count began to fall, usually four to seven hours after the fat meal, changes in the suspension stability of the red blood cells began to develop (fig. 1). First the red blood cells appeared to be slightly adhesive to one another, and here and there a red blood cell stuck momentarily to the wall of a blood vessel or to a white blood cell which was adherent to a vessel wall. The tendency to rouleau formation became more pronounced, the size of these formations increased greatly, and the cells showed a definite tendency to remain together under circumstances which normally would have separated them. Simultaneously the speed of the circulation decreased. This tendency toward aggregation gradually became much greater, and in capillaries and venules red blood cells began to aggregate in irregular small clusters of 4 to 12 or more cells. In many vessels the flow of blood became extremely slow, and frequently the flow stopped completely in some vessels. These changes became first evident and remained most pronounced in the venules.

When the changes just described became pronounced the aggregates of red blood cells assumed an amorphous, homogeneous appearance, and the outlines of the individual cells could no longer be distinguished clearly. Frequently the red blood cells had the appearance of being covered by a surface film. In those vessels in which the rate of flow was greatly reduced, particularly in the venules and smaller veins, the blood flowed in great "chunks" (figs. 3B and C, and 4A and B). When adherent cells separated, one frequently saw them stretched out of shape, and on a few occasions thin viscous strands could be seen attaching them together. At this stage distorted red blood cells were frequently seen, and sometimes clumps of platelets and platelets adhering to red blood cells were observed. Simultaneously with these changes there was observed sometimes a fall in the platelet count as shown in figure 2. As a rule, however, no increased adhesiveness of the white blood cells was noted. Petechial hemorrhages sometimes appeared in those pouches in which adhesiveness of the red blood cells was observed. It is interesting, however, that these hemorrhages occurred after a longer period of exposure and were less numerous than in our control hamsters. Sometimes they were absent for as long as eight hours of exposure in fat-fed animals, whereas they usually appeared within

Fig. 2. This graph represents the average values found in six hamsters with determinations done at regular intervals during the period of observation. One will note a fall in the platelet count in the interval from four to seven hours when aggregation of the red blood cells is present and clumping of the platelets is quite often observed in the intact circulation.
Fig. 3. This figure and the following figure 4 are enlarged frames from the colored motion picture taken of these experiments. Because of the great loss of detail resulting from such enlargement and reproduction the pictures have been retouched in order to make apparent what is obvious in the motion picture.

A. the normal circulation. (1) Venule at lower right joining a vein. These vessels are so large and circulation so rapid that individual cells cannot be seen. (2) Capillary-venular junction. Note the absence of any tendency for the red blood cells to adhere to one another. (3) Capillary junction. Note the absence of red blood cells in the lower right branch. This “plasma skimming” is frequently seen in capillaries. A few moments later this capillary was filled with red blood cells.

B. Hamster no. 57. Fat fed. These three pictures were taken at the same capillary-venular junction at various intervals. (1) Six and one-half hours after the fat meal. Note the pronounced rouleau formation and the small irregular aggregates of red blood cells. The circulation had slowed down considerably. (2) The same area one hour later showing more pronounced aggregation of the red blood cells. The flow of blood had stopped completely except in the larger venule across the top of the picture. (3) The same area two and one-half hours later showing return toward normal. The lipemia had now cleared, the blood was flowing rapidly in all vessels and there was very little tendency for the cells to aggregate, most of them flowing freely and singly.

C. Same Hamster (no. 57). Fat fed. Another area showing: (1) a venule and vein seven hours after fat meal with large aggregates of red blood cells and a very slow circulation, and (2) the same area three hours later showing the absence of “clumping” and a more rapid flow.
three hours in our control animals. Some degree of aggregation and adhesiveness of the red blood cells and slowing of the circulation was observed in all of the hamsters which developed a significant lipemia. This aggregation of red blood cells did not depend on the duration of exposure of the pouch, frequently being present immediately after exposure, and usually appearing within one hour after the pouch was prepared. The degree of aggregation did not appear to depend on the height of the lipemia, as far as we could determine. In general, however, the changes were more pronounced if the animal developed a high lipemia (table 1).

With clearing of the visible lipemia 7 to 10 hours after the fat meal, the circulation became more rapid and the adhesiveness of the red blood cells became less pronounced (figs. 3B and C, and 4A and B). In many vessels in which the circulation had been stopped, the sticky masses of cells were seen slowly oozing out of the vessels into more rapid flowing streams of blood (fig. 4B). The circulation then gradually assumed a more normal appearance. The period of time from the beginning of the increased adhesiveness of the blood elements until the circulation began to revert to normal varied from one and one-half to five and one-half hours with an average of three and one-quarter hours. We have never seen the circulation return entirely to normal in the same pouch during the period of observation, owing in part, probably, to the trauma associated with long exposure of the pouch. However, prolonged exposure, per se, caused no change in the adhesiveness of red blood cells. In several animals, when these circulatory abnormalities had cleared up as completely as possible, examination of the other cheek pouch revealed a normal-appearing circulation. In addition, several hamsters with circulatory changes as marked as those just described were allowed to recover and the same pouch was observed several days later. The circulation was then normal.

C. Circulation in the Cheek Pouch after Injections of Large Molecular Substances

We have confirmed the observation of Thorsen and Hint* that after intravenous injections of small amounts of high molecular weight hydrophilic colloidal solutions of gelatin and dextran (molecular weight, 210,000) changes occur in the suspension stability of the red blood cells similar to those associated with lipemia (fig. 4C). These changes have also been observed by us after intravenous injections of egg albumin and thromboplastin, and by Thorsen and Hint following injection of thrombin and fibrinogen. In each of these circumstances the nature of the circulatory changes appeared similar; the red blood cells became adhesive, aggregated, and the circulation slowed. In these experiments the degree of the circulatory change varied directly with the amount of the substances injected. In all instances the circulatory changes could be reversed toward normal by the injection of low molecular weight dextran (molecular weight, 10,000). The exact mechanism whereby this reversal is effected is

---

**Table 1.—Summary of the Findings in the Nineteen Hamsters Which Form the Basis for the Graph in Figure 1**

<table>
<thead>
<tr>
<th>Hamster</th>
<th>Increased adhesiveness of platelets</th>
<th>Height of lipemia (chylo-micra)</th>
<th>Number of chylo-micra counts</th>
<th>Degree of aggregation of R.B.C.'s</th>
<th>Time after pouch exposure that aggregation appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 17</td>
<td>0+</td>
<td>90</td>
<td>1</td>
<td>Moderate</td>
<td>2½ hours</td>
</tr>
<tr>
<td>No. 20</td>
<td>+</td>
<td>125</td>
<td>4</td>
<td>Pronounced</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 29</td>
<td>-</td>
<td>100</td>
<td>3</td>
<td>Pronounced</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 33</td>
<td>+</td>
<td>210</td>
<td>5</td>
<td>Pronounced</td>
<td>½ hour</td>
</tr>
<tr>
<td>No. 37</td>
<td>-</td>
<td>80</td>
<td>5</td>
<td>Slight</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 45</td>
<td>0</td>
<td>50</td>
<td>1</td>
<td>Slight</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 46</td>
<td>-</td>
<td>100</td>
<td>6</td>
<td>Moderate</td>
<td>1½ hours</td>
</tr>
<tr>
<td>No. 48</td>
<td>-</td>
<td>100</td>
<td>2</td>
<td>Slight</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 51</td>
<td>-</td>
<td>90</td>
<td>6</td>
<td>Pronounced</td>
<td>2½ hours</td>
</tr>
<tr>
<td>No. 53</td>
<td>-</td>
<td>90</td>
<td>4</td>
<td>Moderate</td>
<td>2½ hours</td>
</tr>
<tr>
<td>No. 56</td>
<td>-</td>
<td>125</td>
<td>7</td>
<td>Pronounced</td>
<td>1 hour 40 min.</td>
</tr>
<tr>
<td>No. 57</td>
<td>+</td>
<td>5</td>
<td>4</td>
<td>Pronounced</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 59</td>
<td>+</td>
<td>90</td>
<td>2</td>
<td>Moderate</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 61</td>
<td>+</td>
<td>125</td>
<td>6</td>
<td>Moderate</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 62</td>
<td>-</td>
<td>200</td>
<td>5</td>
<td>Pronounced</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 64</td>
<td>+</td>
<td>250</td>
<td>2</td>
<td>Moderate</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 65</td>
<td>+</td>
<td>50</td>
<td>3</td>
<td>Slight</td>
<td>1 hour</td>
</tr>
<tr>
<td>No. 66</td>
<td>0</td>
<td>90</td>
<td>2</td>
<td>Slight</td>
<td>Immediately</td>
</tr>
<tr>
<td>No. 68</td>
<td>+</td>
<td>90</td>
<td>3</td>
<td>Pronounced</td>
<td>Immediately</td>
</tr>
</tbody>
</table>

In the column showing adhesiveness of the platelets the 0 indicates absence, the (+) signifies that no comment was made on the platelets during the experiment. During most early experiments our interest was primarily in the striking changes of the red blood cells; in later experiments our attention was focused also on the platelets, and one will observe that when special note was made of their condition there was evidence of increased adhesiveness in the great majority.

The degree of aggregation of red blood cells was not dependent on the duration of exposure since in over half the cases aggregation was present immediately after exposure of the pouch and in the majority within one hour.

Exposure, per se, for as long as six hours causes no change in the adhesiveness of the red blood cells.

* The animals which received only a large fat meal on the day of observation; all the other hamsters received a high fat diet for variable periods before in addition to the large fat meal on the day of observation.

---
CIRCULATORY CHANGES DUE TO LIPEMIA

Fig. 4. A. Hamster no. 68. Fat fed. (1 and 2) Two venular junctions with pronounced clumping of the red blood cells seven hours after the fat meal. The blood was flowing very slowly at this time. (3 and 4) Two venular junctions in the same animal three hours later showing a normal circulation again. The aggregation of the blood cells had disappeared and the flow was again of normal rate.

B. Hamster no. 51. Fat fed. (1 and 3) Pronounced clumping of the red blood cells seven and eight hours after the fat meal. In these vessels the flow has almost completely stopped. (2) Note the amorphous mass of hemoglobin with complete loss of cell outlines; this was slowly oozing into the vessel on the left, the red cells remaining adherent for a considerable distance along this latter vessel (at arrow) before breaking off. (4) A vessel from the same animal five hours later with a normal circulation. The cells are seen floating freely and singly and the flow has resumed normal speed.

C. (1, 2, and 3) Aggregation of the red blood cells following intravenous injection of large molecular weight dextran (molecular weight 210,000). The flow was very slow. (4) A similar picture after intravenous gelatin. Note the similarity of this clumping to that produced by a high fat meal.

unknown. It does not appear to be due only to the diluting effect of the low molecular weight dextran solution because injections of an equal amount of Ringer’s solution had no effect on the aggregated red blood cells or the rate of the blood flow.

In four of the hamsters which had circulatory changes associated with alimentary lipemia low
molecular weight dextran was injected. Two of these showed definite but not striking reversal of the circulation toward normal. In some vessels the circulation was resumed and in general the rate of flow increased. In the remaining two the effect was equivocal. In general the degree of improvement of the circulation which followed the injection of low molecular weight dextran in fat-fed animals was much less pronounced than was observed in those animals whose circulatory changes were due to high molecular weight substances.

D. Study of the Brains after Trypan Blue Injection

Of the 24 brains studied following intravenous injection of trypan blue, abnormalities were found only in those which had received high molecular weight dextran. All six of these showed extravasation of the dye into the brain, in three it was slight and in the other three it was pronounced with diffuse staining of the brain (fig. 5). The veins and arteries appeared equally affected, and the cortical as well as the subcortical vessels showed abnormalities. There was not a very complete correlation of the duration of the circulatory changes with the severity of extravasation of the dye.

In the 11 control hamsters and the 7 hamsters which had shown aggregation of the red blood cells due to a high fat meal there was observed no extravasation of the trypan blue. Although all these fat-fed hamsters showed definite aggregation of the red blood cells, and in several of these the clumping was what we considered pronounced, in no case was it as severe as we produced in all the hamsters which received dextran and which exhibited changes in the blood-brain barrier.

Discussion

The underlying mechanism of the circulatory changes produced by a high fat intake is not clear. Previous darkfield microscopic observations\(^4\) and the cinephotomicrographs taken in the present study leave no doubt that an adhesive envelope develops around the red blood cells. In all likelihood this is the principal cause of the aggregation of the red blood cells and of the slowed circulation. The observations of Swank, Franklin and Quastel\(^5\) that changes do occur in the paper chromatographic pattern
of the plasma proteins following fat meals and approximately at the time that the circulatory changes occur can be considered collateral and relevant data although not proof that the plasma proteins take part in this mechanism.

It was previously suggested⁴ that this increased adhesiveness and aggregation of the red blood cells was due to competition of the chylomicra with the red blood cells for a substance or substances which maintain a stable suspension of the blood elements. The chylomicra which appear quickly and have a large surface area relative to their mass may, through this competition, produce a temporary deficiency of these substances and in this way cause the development of an adhesive surface film on the particulate matter in the blood. This is supported by the observation that the chylomicra as well as the red blood cells and platelets show a simultaneous tendency to aggregate. The development of this adhesive envelope when the visible chylomicra are diminishing in number could be due to a breakdown of the chylomicra into smaller and smaller particles prior to their removal from the circulation.¹⁰ In this way they would require a greater amount of the hypothetic “emulsifying agent” since their total number and their surface area would have increased. This hypothesis is supported by the fact that the visible chylomicra in the S₁ range of 100 to 40,000 contain approximately 7 per cent protein presumably located as a film on their surface, whereas the invisible fat in the S₁ 4 category contain approximately 25 per cent protein.¹¹ It might also be added that R. J. Rossiter has noted that when fat containing radioactive iodine is fed to dogs the peak of the radioactive fat in the plasma occurs concomitantly with the peak in concentration of fat in the plasma. In the red blood cells, however, the peak of radioactivity occurs later, and when the concentration of fat in the plasma is decreasing.*

The changes in the circulation produced by intravenous injection of substances of large molecular weight appeared similar in nature to, although they were usually more pronounced than, those produced by alimentary lipemia. In the process of development of these changes Thorsen and Hint⁶ observed that a surface film developed on the red blood cells when the concentration of the substances of high molecular weight reached a critical level. This surface film formed from the suspension fluid, and it disappeared when the red blood cells were resuspended in saline. In our studies with high molecular weight substances we observed this surface film and often saw thin viscous strands connecting cells together. We were able, as were also Thorsen and Hint, to return the circulation to normal by injection of low molecular weight dextran. Our studies were otherwise not helpful in explaining the mechanism of the changes after injections of substances of high molecular weight. Similar surface films and viscous strands connecting red blood cells were also noted after fat feeding, but the injection of dextran of low molecular weight in these animals was either ineffective (two cases) or less effective (two cases) in returning the circulation to normal.

The effect of the surface film changes upon the red blood cells was to slow the circulation to the point that the flow was often stopped in many vessels. This slowing was presumed to be associated with an increase in the viscosity of the blood. Recent measurements of viscosity of the blood in hamsters after fat meals have shown that the viscosity is always increased when adhesiveness of the red blood cells is evident, and an increase of 50 to 100 per cent in the viscosity is not unusual.†

That these circulatory changes, when severe enough, can produce pathologic alterations in the blood-brain barrier has been demonstrated in the animals which received dextran of high molecular weight. The barrier was not noticeably affected in the animals receiving fat meals probably because the circulatory changes were less marked. Perhaps repeated embarrassments of the circulation, which could be expected to occur in subjects susceptible to circulatory changes after fatty meals, or the combination of these changes with other factors such as endothelial damage, an already deficient circulation, or other factors altering the suspension stability of the blood would lead to tissue hypoxia and ultimate pathologic changes. It is

* Personal communications.

† Unpublished observations by one of us (R. L. S.).
well known that the central nervous system is extremely sensitive to hypoxia. The effects of hypoxia due to slowing of the cerebral circulation at the capillary and precapillary level have been demonstrated by Swank and Hain.\textsuperscript{13}

The significance of the circulatory changes which were observed after fat ingestion and their relationship to multiple sclerosis is not clear. Data indicating that the lesions of multiple sclerosis are related to the vascular system, and in particular to veins, is readily available. Rindfleish in 1863\textsuperscript{14} and Ribbert in 1882\textsuperscript{15} regarded circulatory disturbances as a factor in the production of the disease. In recent years Putnam’s well known venous thrombosis hypothesis\textsuperscript{15-21} has received considerable attention. However, many workers have been unable to find thrombosed veins in plaques. Dow and Berglund\textsuperscript{22} studied 60 lesions in five patients and found that 40 of them were located about a central vein, but a thrombus was present in only six of these. Fog\textsuperscript{23} in a study of the topographic distribution of the plaques in the spinal cord found a perivenous localization in all cases but found no evidence of thrombosis in these veins. Scheinker\textsuperscript{24, 25} studied the early stages of plaque formation in 20 cases of multiple sclerosis. He found that most of these were perivascular, the vessel in most instances being a small, centrally located vein. He frequently observed agglutinated red blood cells in these vessels. Thus the majority opinion is that the lesions form around central veins, but the role that thrombosis of these veins plays in the pathogenesis of the disease is clouded by inconsistent data.

Evidence is also available that vascular abnormalities in patients with multiple sclerosis are not limited to the central nervous system. Brickner and Franklin\textsuperscript{26} observed spasms of the retinal arteries during transient scotomata, and Rucker\textsuperscript{27} described sheathing of the retinal veins in patients with multiple sclerosis. Tortuous and irregular blood vessels have been observed in the nail beds by several workers.\textsuperscript{28, 29} Shulman and co-workers\textsuperscript{30} reported capillary fragility, and Grain and Jahsman\textsuperscript{31} noted many spastic phenomena in blood vessels of the extremities.

Other phenomena related to the blood itself have been found in patients with multiple sclerosis. These include decreased platelet counts during clinical activity of the disease,\textsuperscript{32} increased platelet adhesiveness during exacerbations,\textsuperscript{33} and changes in the plasma proteins shown by paper chromatography,\textsuperscript{34, 35} by ultracentrifuge studies,\textsuperscript{36} and by electrophoresis.\textsuperscript{37} Finally “sludging” of the blood has been reported by Kniseley and co-workers\textsuperscript{38} and by Roizin, Abell and Winn\textsuperscript{39} in patients with multiple sclerosis.

If it could be determined that the “sludging” described by these workers is more than simple aggregation without adhesiveness this phenomenon could be of importance in multiple sclerosis. “Sludging” of the blood has been observed by Kniseley and his colleagues in many conditions, and has been produced experimentally in animals by the injection of vasoconstrictor substances and by the stimulation of vasoconstrictor nerves.\textsuperscript{40} It should be realized that the presence of “sludged” blood in man has been determined under conditions which do not permit clear visualization of the blood elements because of the limited magnification possible, and that the classic rouleau formation of Fahraeus\textsuperscript{41} has been confused with the “sludged” blood concept by many investigators as pointed out by Lutz.\textsuperscript{42} To the authors it seems imperative that an increased adhesiveness of the red blood cells with significant slowing of the blood flow must be clearly shown before “sludged” blood can be accepted as a factor in the pathogenesis of multiple sclerosis or any other disease. Also, it will be necessary to prove that a large fat intake will produce adhesiveness and intravascular aggregation of the red blood cells in man before this mechanism can be seriously considered as pathogenetic. Previous studies in humans\textsuperscript{43} have shown that when blood is examined in vitro there is increased adhesiveness of the red blood cells six to nine hours after a large fat meal. If the hamster’s blood is examined in vitro these changes are also seen when, and only when, there is increased adhesiveness and aggregation observed in the intact circulation. This would indicate that intravascular changes in the adhesiveness of the blood elements do occur in the human after high fat meals. To establish
this point adequately may be very difficult because of the limitations encountered in viewing capillary blood flow with adequate illumination and magnification in man.

A high fat diet has long been thought to increase the coagulability of the blood. Moolton and colleagues have shown that a diet rich in animal fat causes a distinct rise in the adhesiveness of the platelets and in some cases in the count as well. He has isolated a lipid substance, present in all fatty tissue, which when injected increases the platelet count and adhesiveness and the coagulability of the blood. The efficacy of a high fat diet in relieving bleeding disorders has been reported, and a diet poor in fat has been utilized to combat the thrombotic tendency in surgical patients and in patients following coronary thrombosis. Of interest also is the reported decrease in vascular thrombosis in Norway during the recent war when the fat intake was reduced by about 50 per cent. The relationship of the changes which we have described following a large fat meal to the coagulation mechanism is unknown. The aggregation of the blood elements following a large fat meal or the intravenous injection of gelatin and dextran appeared the same as those observed by us and by Thorsen and Hint following the intravenous injections of thromboplastin, thrombin and fibrinogen. Whether the circulatory changes associated with alimentary lipemia can, when severe enough or when combined with other factors, lead to thrombosis remains to be determined.

Although there is considerable evidence to indicate that the incidence of multiple sclerosis varies with the dietary fat intake, we do not maintain that this is the basic cause of the disease. We do feel that there is evidence to indicate that a high fat diet is an important and common precipitating or accelerating factor in individuals susceptible to the disease. The nature of the basic defect causing susceptibility is unknown. If this is true the present work demonstrates a possible mechanism whereby a high fat diet might be an important factor in the pathogenesis of multiple sclerosis as well as vascular thrombosis.

Summary

Following meals high in fat content changes occur in the circulation of the hamster. These changes consist of an increased adhesiveness and aggregation of the red blood cells, and sometimes clumping of the platelets, accompanied by slowing and even at times complete cessation of flow of the blood. These changes appear after the peak of the lipemia has been passed and develop to their maximum as the lipemia clears. After complete clearing of the lipemia the suspension stability returns toward normal and the circulation again assumes a more normal appearance. Similar circulatory changes can be produced by the injection of solutions of large molecular weight. These circulatory alterations when severe enough cause pathologic changes in the blood-brain barrier. The significance of these findings and their possible relationship to multiple sclerosis and vascular thrombosis are discussed.

NOTE: A 20 minute, 16 mm. colored cinephotomicrograph with titles has been prepared which shows the circulatory changes described in this paper.

SUMARIO ESPAÑOL

Observaciones hechas en la circulación intacta del caballo revelaron que luego de una comida grasosa los eritrocitos mostraron un aumento en la tendencia a adherirse y agregarse y algunas veces aglutinación de plaquetas, acompañado por un retardo en la circulación. Estos cambios son reversibles, la circulación re-asumiendo su apariencia normal con desaparición completa de la lipemia. Cambios circulatorios similares pueden ser producidos mediante la inyección de soluciones de moléculas grandes. Estas alteraciones circulatorias cuando de un grado marcado, causan cambios patológicos en la barrera cerebro sanguínea. El significado de estos hallazgos y su posible relación a la esclerosis múltiple y trombosis vascular se discute.

REFERENCES


CIRCULATORY CHANGES DUE TO LIPEMIA


Intravascular Aggregation and Adhesiveness of the Blood Elements Associated with Alimentary Lipemia and Injections of Large Molecular Substances: Effect on Blood-Brain Barrier

CHESTER F. CULLEN and ROY L. SWANK

Circulation. 1954;9:335-346
doi: 10.1161/01.CIR.9.3.335

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1954 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/9/3/335

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/